

# **Human Papillomavirus Genotyping and Cervical Smear in Duhok/Iraq**

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# Introduction

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- **Papillomaviruses** comprise an ubiquitous group of viruses known to infect mammals, birds and reptiles, with species and tissue-specificity.
  - The human type (HPV), which targets the stratified squamous epithelia, is one of the most common sexually transmitted infections for both men and women worldwide.
  - This infection is an essential cause of cervical cancer.

# Aims of the study

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- 1. Determine HPV genotypes in Duhok/Iraqi women.**
- 2. Identify the distribution of HPV genotypes in different Pap smear readings**

***MATERIALS***

***&***



***METHODS***



During a period extended from:

November-2015 ----- September-2016

A total of 64 married women (17-60 Y) who attended the cytologic clinical centers in Duhok/Iraq, were enrolled in the study.

# Two samples were taken from each woman

**HPV-DNA**

- From endocervical canal with sterile cytobrushes, rinsed in transport buffers & then stored at 2-8 °C

**Pap smear**

- From transitional zones, then processed in ThinPrep liquid based devices & stained with Pap stain

## PCR preparation

Using the QIAamp Mini Kit (Qiagen, Hilden, Germany), extraction of DNA was performed according to the manufacturer's instruction.

For each sample two amplification reactions were run

One with medium-high risk primers

While the other with low risk primers.

PCR Premix and Taq reagents are common to both reactions





PapillomaStrip **kit** used was based on the reverse blot technique that allows qualitative detection of 37 anogenital HPVs DNA samples.

These *Papillomavirus* subtypes comprise:

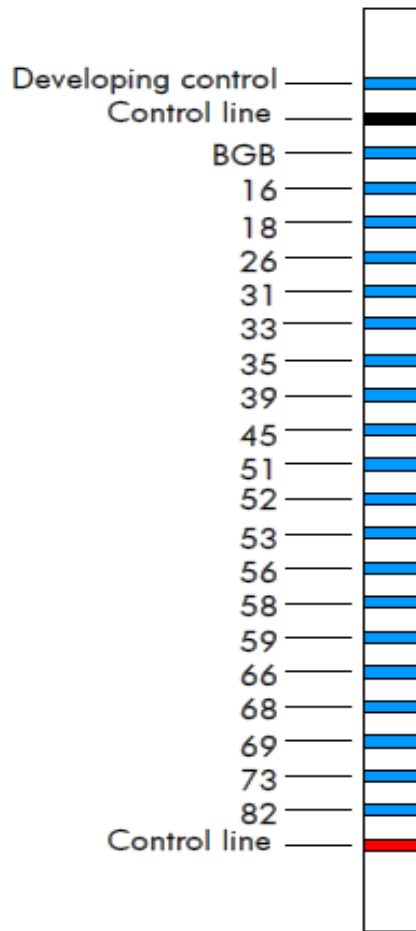
- **Medium-high risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73 & 82**
- **Low risk: 6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 70, 71, 72, 74, 81, 83, 84 & 91.**

# Thermocycling (PCR program used for amplification of HPV genes) included:

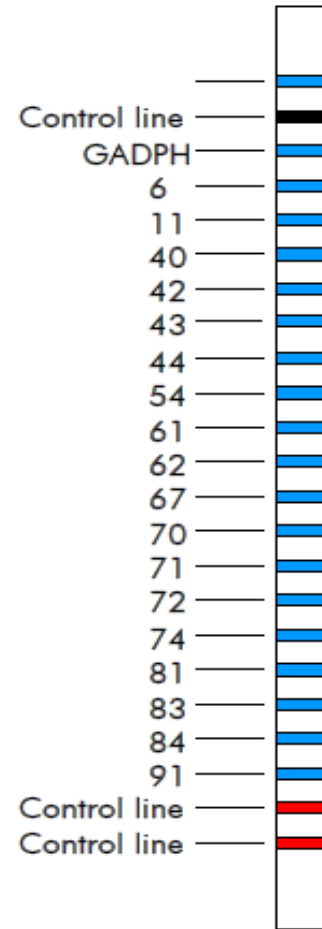
	Step	Temp.	Time	NO. Of cycle
1.	Initial denaturation	94 °C	5 min	1
2.	Denaturation	94 °C	1 min	40
3.	Annealing	58 °C	1 min	
4.	Extension	72 °C	1 min	
5.	Final extension	72 °C	5 min	1
6.	Holding step until samples removed	3. °C		

# Interpretation of Results:

## HIGH PAPILOMASTRIP



## LOW PAPILOMASTRIP

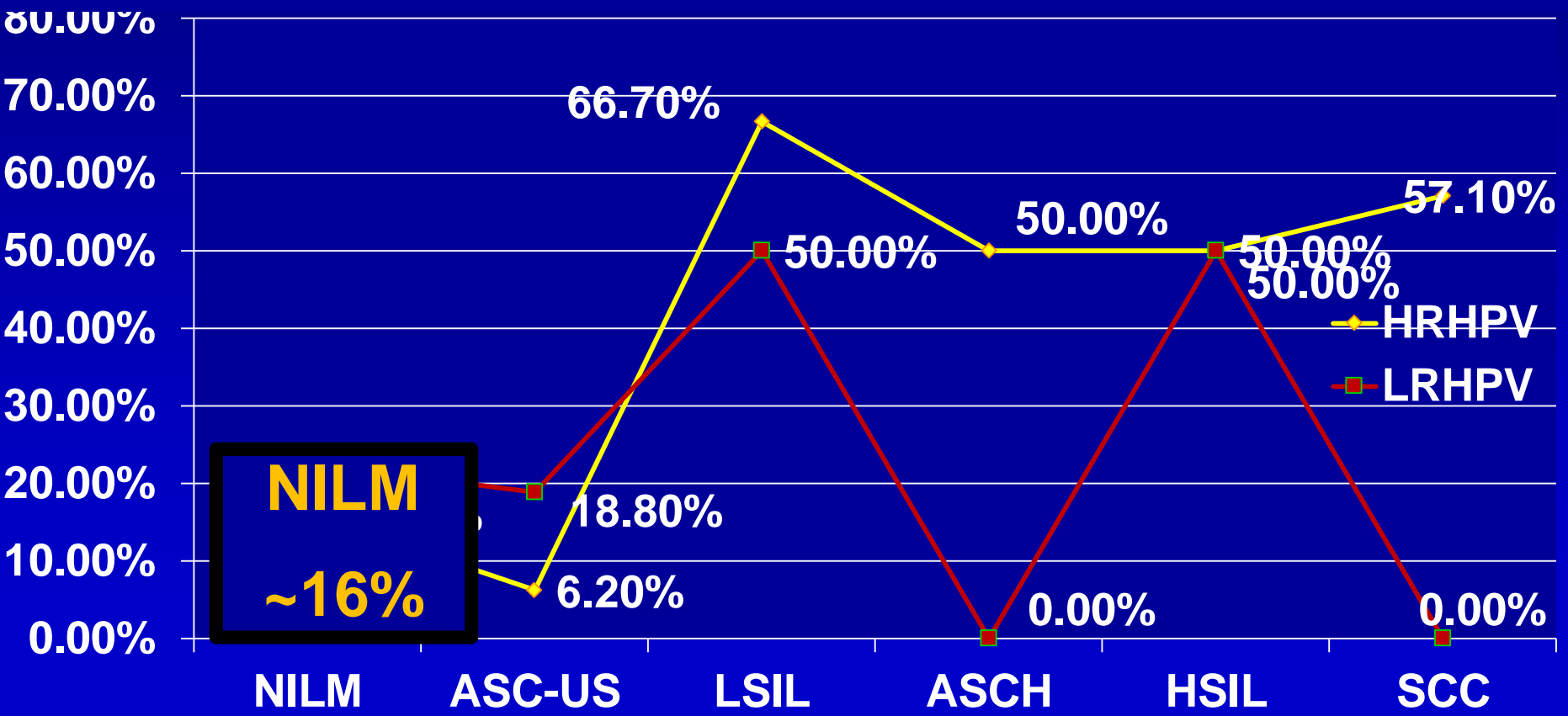


***RESULTS***  
***&***  
***DISCUSSION***



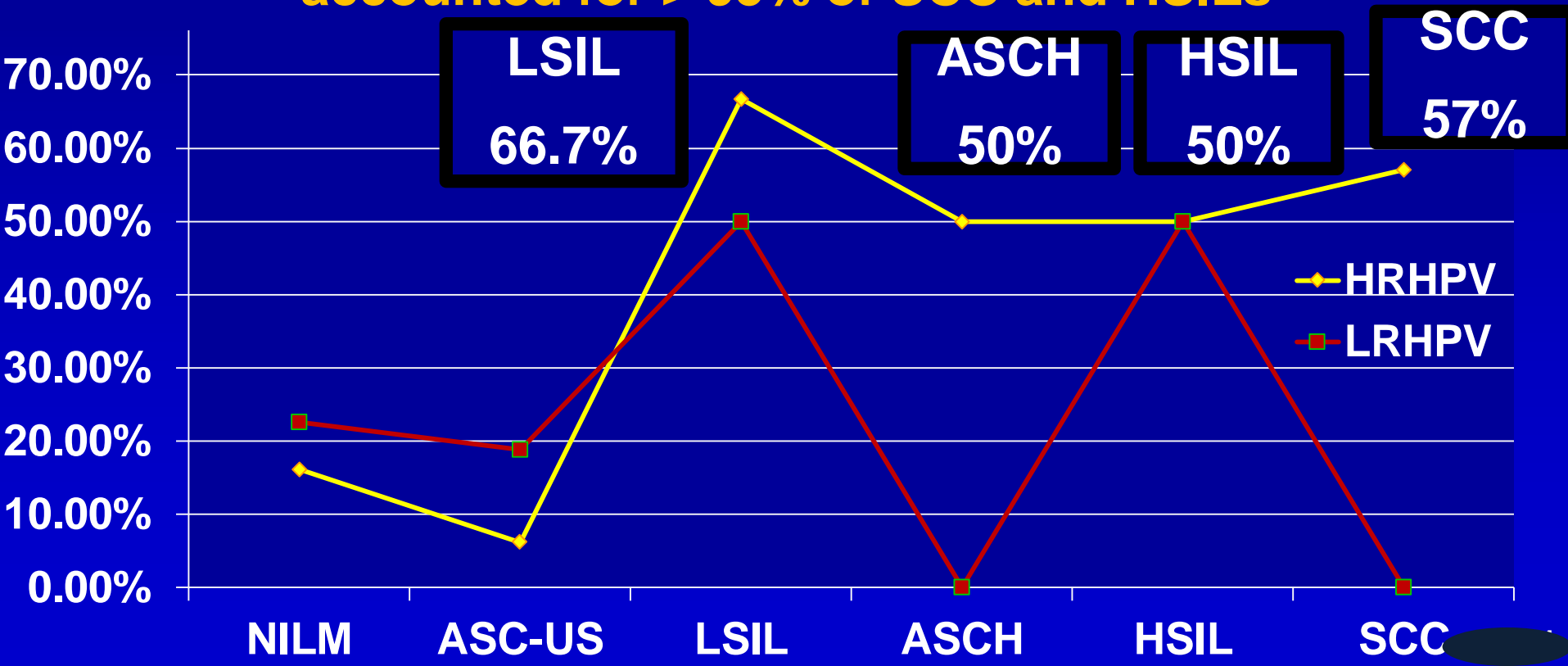
# HPV was detected in 40%

HR-HPVs were observed in ~16% of NILM Pap smears. This ascertains the fact that HPV infection can remain silent for a variable period before a serious lesion will develop & may represent the footprints for a serious clinical implication.

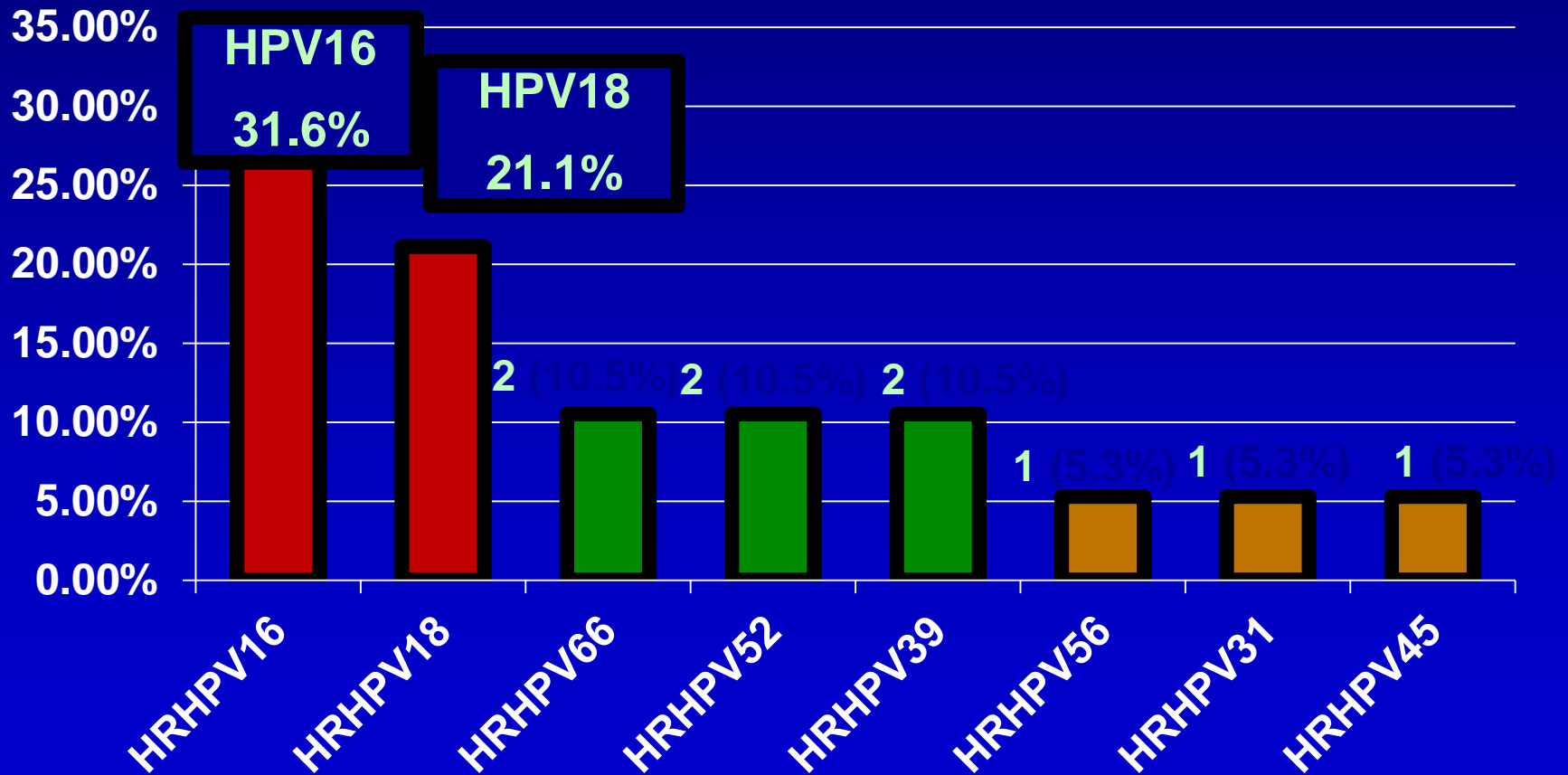


**Among cytologically abnormal smears, there was evident increased HR-HPVs with increasing disease severity with a peak at LSIL level, then remained plateau.**

**However, our HR-HPV burden rates are considered low when compared with what has been reported among unselected Ethiopians and unscreened Kuwait's women where HR-HPV accounted for > 95% of SCC and HSILs**



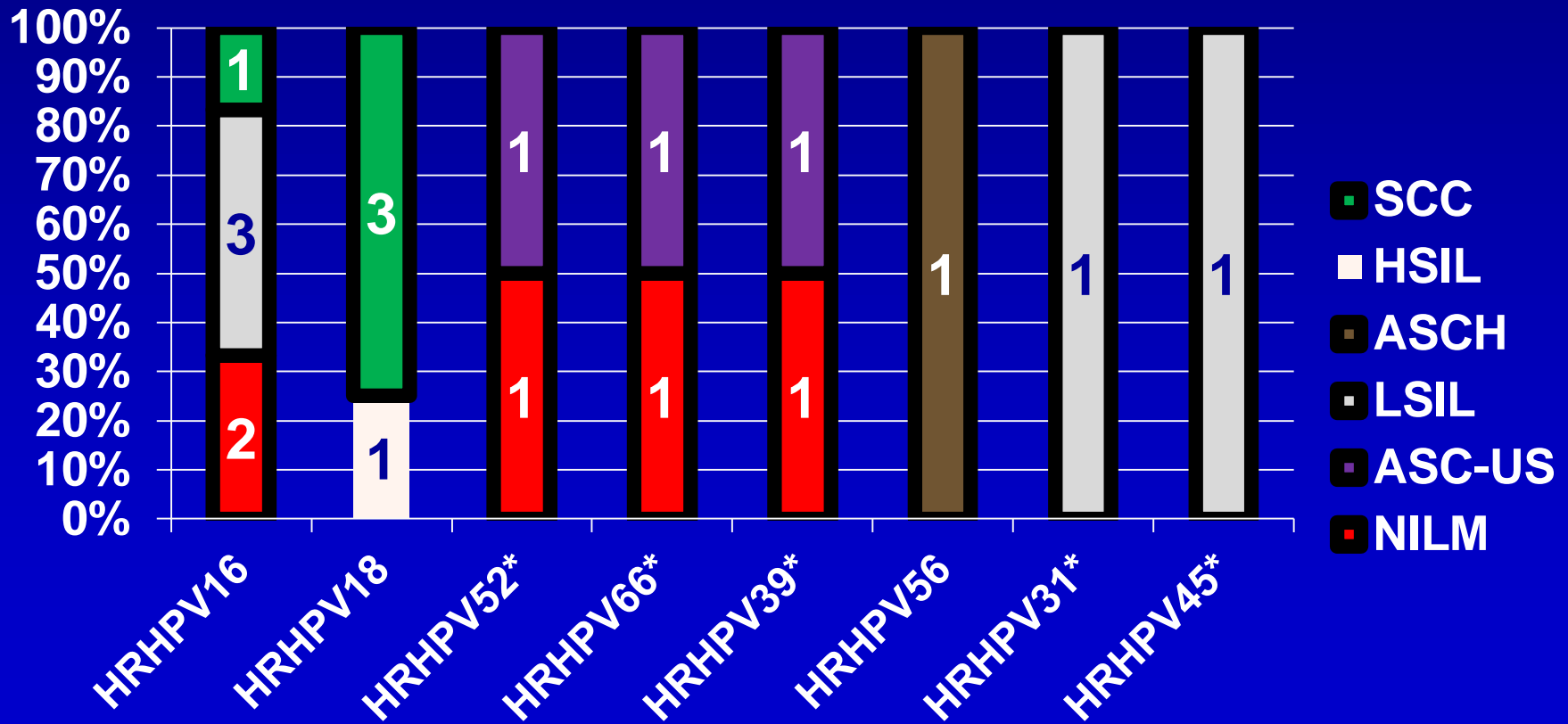
HPV16 formed the most frequent HR followed by HPV18. Other HR-HPV strains identified included HPV52, HPV66, HPV39, HPV56, HPV31 and HPV45.



**HPV16 was identified in NILM, LSIL & malignant smears.**

**While HPV18 strains were exclusive to the SCC and HSILs.**

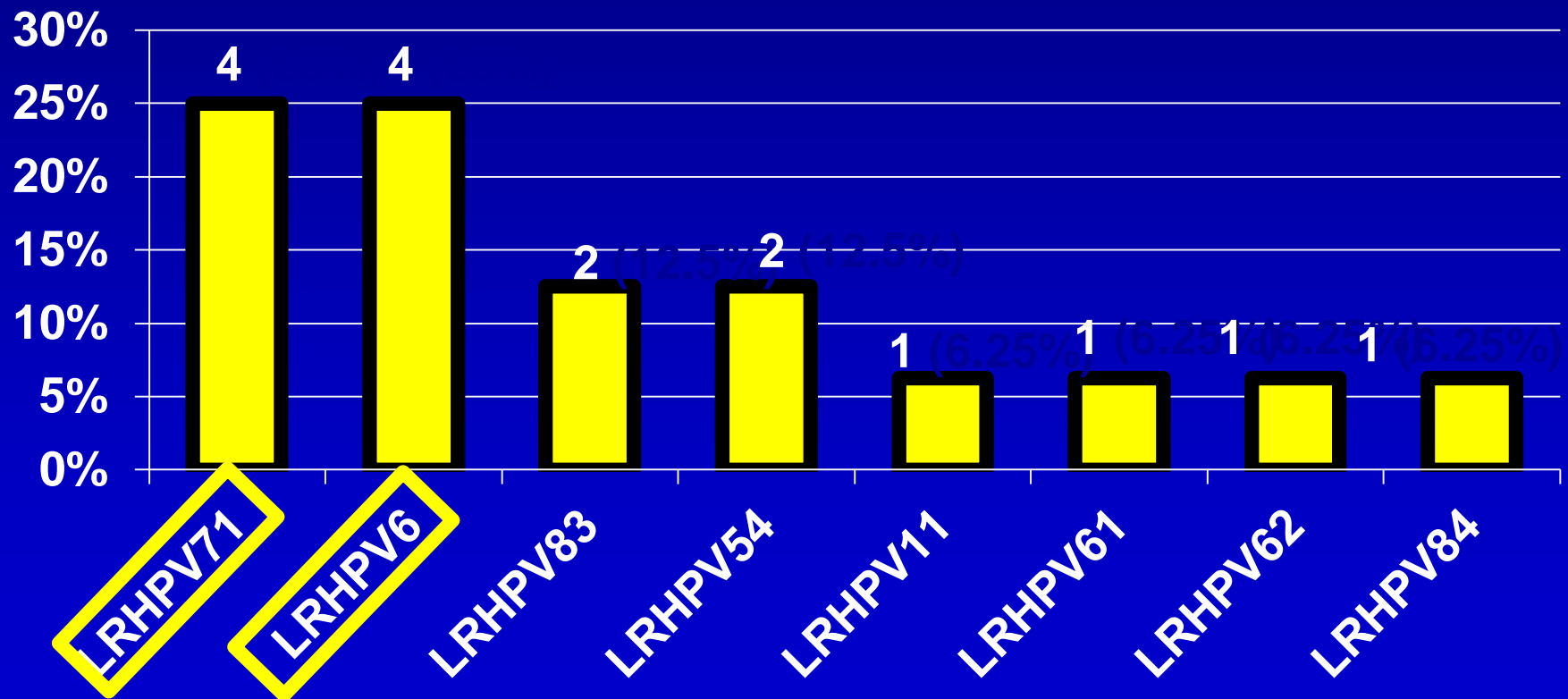
**Certain HR types, like HPV52, HPV66, HPV56, HPV39, HPV31 and HPV45, were more likely to be associated in LSIL and lower cytologic abnormalities and in negative cases, rather than HSIL and SCC**





# LR-HPV

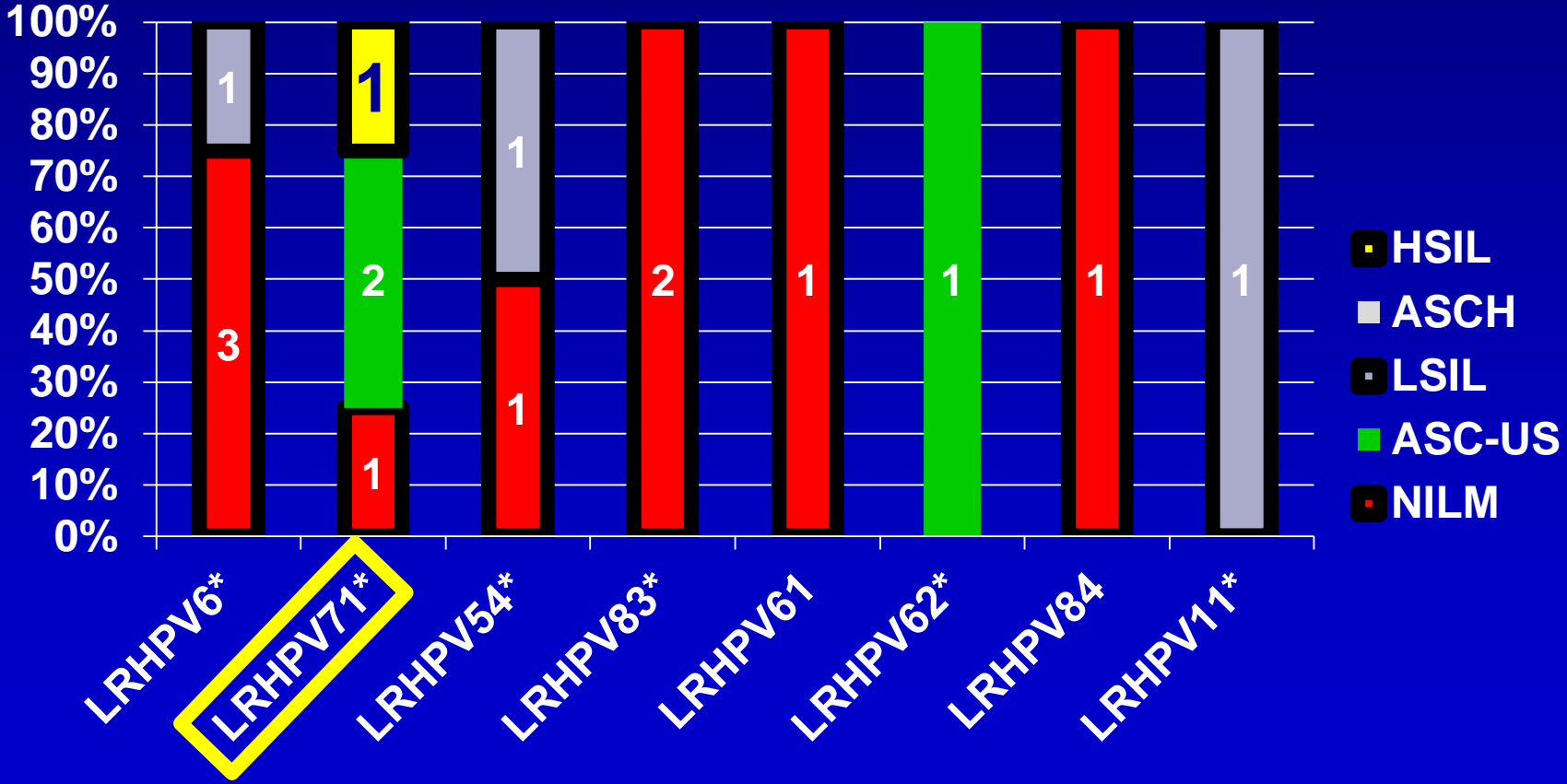
HPV71 & HPV6 formed the commonest genotypes



**Only HPV71 was detected in HSIL in addition to ASC-US, & NILM.**

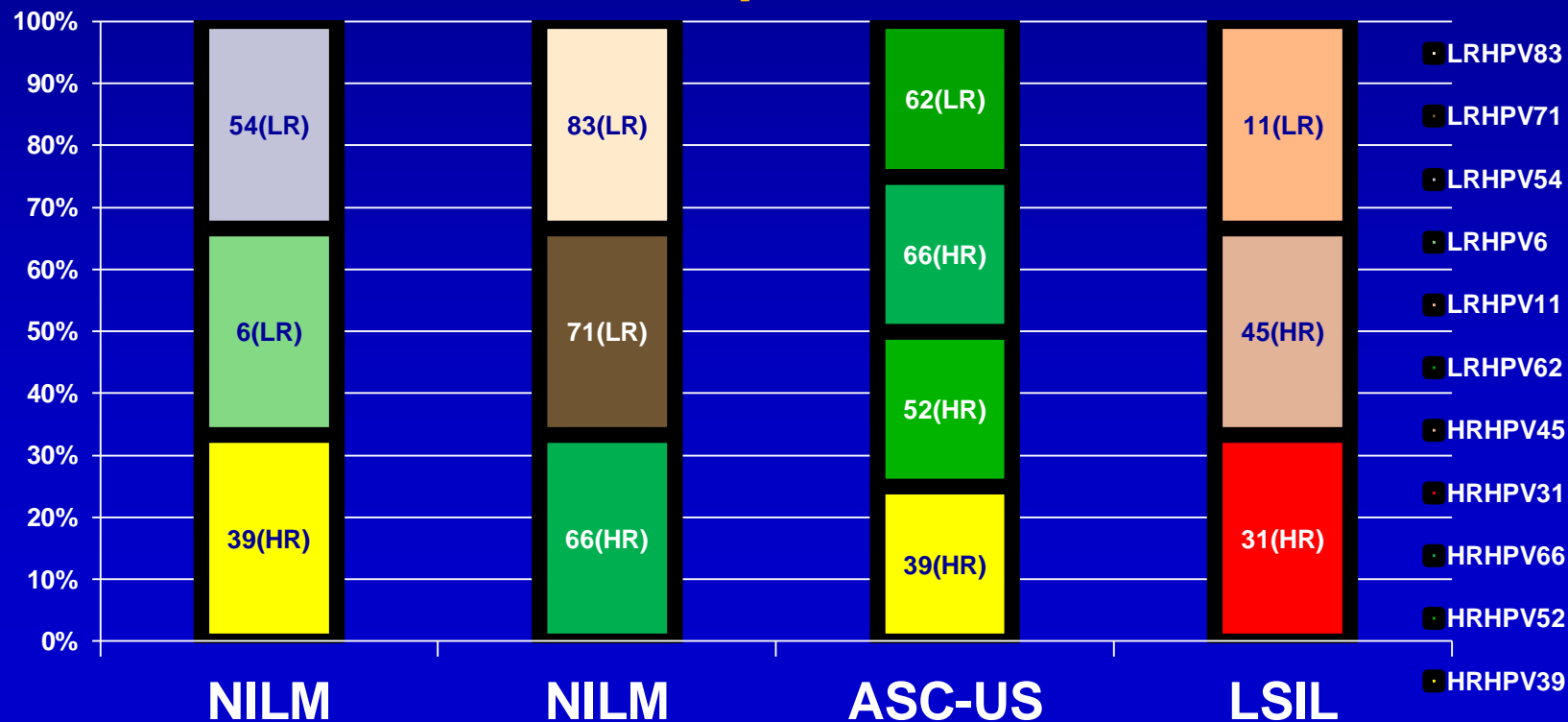
The remaining LR-HPV genotypes were identified in LSIL, equivocal & lower Pap readings.

We failed to identify any LR-HPV among SCC cases



The proportion of **Mixed HPV Infection** in the present study, 4 (15.3%), appears to be much lower than what has been reported by others who restricted their studies on women complaining of difficult infections resistant to antibiotics & reproductive age groups which are expected to be sexually more active & thus harbor more mixed infections.

**Higher grades cervical lesions (HSIL, SCC and even ASCH) tended to harbor single (high or low risk) rather than multiple strains.**



# Conclusions

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- 1. The higher frequency of HR-HPV than the LR-HPV with identification of 4 mixed cases a strongly indicates that our women are at risk of developing cervical cancer and thus vaccination program has to be launched.**
- 2. HPV 16/18 genotypes contributed the highest proportion of the overall HR-HPV with rising frequency among increasing severity of cervical cytologic abnormality.**

# Conclusion...cont..

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3. Detection of HR-HPV in ~ 16% of NILM and ~ 19% of ASC-US smears with restriction of some strains to these 2 categories highlights the great value of HPV genotyping as a surrogate virology test to pick up unscreened women at risk of developing cervical cancer particularly in absence of a proper screening program.

# THANK YOU

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